Determining the pharmacokinetics of nicotinic drugs in the endoplasmic reticulum using biosensors.

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Public Summary:
Nicotine dependence is thought to arise in part because nicotine permeates into the endoplasmic reticulum (ER), where it binds to nicotinic receptors (nAChRs) and begins an "inside-out" pathway that leads to up-regulation of nAChRs on the plasma membrane. However, the dynamics of nicotine entry into the ER are unquantified. Here, we develop a family of genetically encoded fluorescent biosensors for nicotine, termed iNicSnFRs. The iNicSnFRs are fusions between two proteins: a circularly permutated GFP and a periplasmic choline-/betaine-binding protein engineered to bind nicotine. The biosensors iNicSnFR3a and iNicSnFR3b respond to nicotine by increasing fluorescence at [nicotine] <1 microM, the concentration in the plasma and cerebrospinal fluid of a smoker. We target iNicSnFR3 biosensors either to the plasma membrane or to the ER and measure nicotine kinetics in HeLa, SH-SY5Y, N2a, and HEK293 cell lines, as well as mouse hippocampal neurons and human stem cell-derived dopaminergic neurons. In all cell types, we find that nicotine equilibrates in the ER within 10 s (possibly within 1 s) of extracellular application and leaves as rapidly after removal from the extracellular solution. The [nicotine] in the ER is within twofold of the extracellular value. We use these data to run combined pharmacokinetic and pharmacodynamic simulations of human smoking. In the ER, the inside-out pathway begins when nicotine becomes a stabilizing pharmacological chaperone for some nAChR subtypes, even at concentrations as low as approximately 10 nM. Such concentrations would persist during the 12 h of a typical smoker's day, continually activating the inside-out pathway by >75%. Reducing nicotine intake by 10-fold decreases activation to approximately 20%. iNicSnFR3a and iNicSnFR3b also sense the smoking cessation drug varenicline, revealing that varenicline also permeates into the ER within seconds. Our iNicSnFRs enable optical subcellular pharmacokinetics for nicotine and varenicline during an early event in the inside-out pathway.

Scientific Abstract:
Nicotine dependence is thought to arise in part because nicotine permeates into the endoplasmic reticulum (ER), where it binds to nicotinic receptors (nAChRs) and begins an "inside-out" pathway that leads to up-regulation of nAChRs on the plasma membrane. However, the dynamics of nicotine entry into the ER are unquantified. Here, we develop a family of genetically encoded fluorescent biosensors for nicotine, termed iNicSnFRs. The iNicSnFRs are fusions between two proteins: a circularly permutated GFP and a periplasmic choline-/betaine-binding protein engineered to bind nicotine. The biosensors iNicSnFR3a and iNicSnFR3b respond to nicotine by increasing fluorescence at [nicotine] <1 microM, the concentration in the plasma and cerebrospinal fluid of a smoker. We target iNicSnFR3 biosensors either to the plasma membrane or to the ER and measure nicotine kinetics in HeLa, SH-SY5Y, N2a, and HEK293 cell lines, as well as mouse hippocampal neurons and human stem cell-derived dopaminergic neurons. In all cell types, we find that nicotine equilibrates in the ER within 10 s (possibly within 1 s) of extracellular application and leaves as rapidly after removal from the extracellular solution. The [nicotine] in the ER is within twofold of the extracellular value. We use these data to run combined pharmacokinetic and pharmacodynamic simulations of human smoking. In the ER, the inside-out pathway begins when nicotine becomes a stabilizing pharmacological chaperone for some nAChR subtypes, even at concentrations as low as approximately 10 nM. Such concentrations would persist during the 12 h of a typical smoker's day, continually activating the inside-out pathway by >75%. Reducing nicotine intake by 10-fold decreases activation to approximately 20%. iNicSnFR3a and iNicSnFR3b also sense the smoking cessation drug varenicline, revealing that varenicline also permeates into the ER within seconds. Our iNicSnFRs enable optical subcellular pharmacokinetics for nicotine and varenicline during an early event in the inside-out pathway.

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